

Vegetative propagation of the threatened East African yellowwood (*Podocarpus falcatus*)

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Rooting in *Podocarpus falcatus* (Thunb.) Mirb. (syn. *P. gracilior* Pilg.) was studied using branch cuttings and whole seedlings harvested from 3-month-old, 2-year-old, 4-year-old and 8-year-old stockplants. The study was conducted in two separate propagators (two blocks) in which six different IBA treatments, viz. 0µg, 20µg, 40µg, 80µg, 160µg or 320µg IBA/cutting were administered to each of the 50–60 cuttings per treatment. The study found that cuttings from 3-month-old and 2-year-old stockplants responded well to IBA dosages between 20µg and 80µg/cutting, but were inhibited at higher concentrations. Treatments higher than 40µg/cutting significantly ($P < 0.01$) inhibited rooting in cuttings derived from the 4-year-old and 8-year-

old stockplants. Rooting was significantly ($P < 0.01$) better in cuttings derived from 3-month-old and 2-year-old stockplants than from 4-year-old and 8-year-old stockplants. Indolebutyric acid significantly ($P < 0.01$) shortened the time taken for 50% rooting-response in cuttings derived from 3-month-old and 2-year-old stockplants. Notwithstanding the poor rooting response, cuttings derived from 4-year-old and 8-year-old stockplants produced far more roots per cutting than those from 3-month-old and 2-year-old stockplants. Stecklings (plants derived from rooted cuttings) grew significantly ($P < 0.01$) faster than seedlings, but plagiotropism in the former remained a persistent problem.

Introduction

Podocarpus falcatus (Thunb.) Mirb. (syn. *P. gracilior* Pilg.), commonly known as podo or East African yellowwood, is a graceful evergreen tree belonging to the Podocarpaceae family (Geldenhuys 1993, Negash 1995, 2002a, 2003a). Although relatively uncommon compared to *P. latifolius*, the species grows to large size with a wide range of habitat tolerance (Geldenhuys 1993, 1994, Negash 2002a, 2003a).

P. falcatus is an extremely valuable tree with a variety of ecological and environmental services. Its massive evergreen plant body is particularly suited to protecting the soil from the stormy and erosive rainfall frequent on the watersheds where the species occurs. As a result, podo forests contribute greatly to the formation of cool and clear springs as well as to the existence of cool and refreshing habitats, even during the driest and hottest seasons (Negash 1995). Many birds and mammals, including bats and the rare colobus monkey (*Colobus polykomos*), depend on the fruits of this species as their source of food (Geldenhuys 1993, Negash 1995, 2002a, 2003a). Also, the evergreen leaves and the massive branches of the tree serve as a habitat for a variety of organisms.

The yellowish-white wood of *P. falcatus* is resinless, odourless and fine-grained, thus yielding good quality timber for manufacturing panel framing and panels, bakery boards,

cupboards, match-sticks, shelves or fittings where a bright, clean-coloured wood is desirable (Dale and Greenway 1961). If planting is done at appropriate intervals, individual plants can develop into tall and straight timber trees suitable for industrial purposes, but photo-inhibition can be formidable if seedlings are planted in the open field (Negash 1995).

In Ethiopia, this valuable tree species is being threatened as it has been selectively logged for many decades up to now (Russ 1944–1947). On top of this, frequent cultivation of land beneath tree canopies and the practice of tree lopping have resulted in diminished capacity of the species for fruit production and seed fertility (Negash 2003a).

It has been accepted that vegetative propagation of plants is a useful tool for capturing genetic variation in tree improvement and multiplication programmes (Davis and Haissig 1994, Howard 1994, Leakey *et al.* 1994, Negash 2003b). Ritchie (1994) has recommended vegetative propagation as a powerful means of exploiting genetic gains through the capture of both additive and non-additive genetic variance. Talbert *et al.* (1993) have reviewed the benefits and risks of vegetative propagation and concluded that the technology has the potential to create a revolution in forestry practice. Leakey *et al.* (1990) and Leakey *et al.* (1992) emphasised the potential of vegetative propagation in the

domestication of tropical trees.

In this paper, we report a method for the vegetative propagation of *P. falcatus* through the rooting of leafy branch cuttings as an alternative to propagation of the species by seed (Negash 1992, 1995). The importance of propagating conifers by vegetative means has been reviewed by Talbert *et al.* (1993).

Materials and Methods

Stockplant management

Fruits of *P. falcatus* were collected from a stand of trees in central Ethiopia (8°06'N, 39°15'E) on different occasions, and seedlings were raised from *in vitro* germinated seeds following the method described by Negash (1992, 1995). The oldest *Podocarpus* stockplants (8-year-old) were planted within the Science Faculty campus of the Addis Ababa University in 1992, and the next oldest (4-year-old) were planted in 1996 in the same locality. Three-month-old stock seedlings were raised by transplanting *in vitro*-germinated seeds into bottom perforated pots (mouth diameter, 20cm; height, 20cm) while the 2-year-old stockplants were grown in bottom-perforated polyethylene bags (diameter, 15cm; height, 30cm), filled with a mixture of soil, horse dung and sand in a ratio of 2:1:1 (v/v/v). The stockplants were allowed to grow in a glasshouse by equally watering them once a day. Sizes of the four categories of stockplants at the time of cutting harvest as well as the corresponding cutting characteristics are provided in Table 1. The mean minimum and maximum temperatures of the glasshouse ranged from 12 ± 2°C (nights) to 28 ± 3°C (days), respectively. The mean quantum flux density was 750 ± 50 μmol m⁻² s⁻¹ (measured using a LI-COR Quantum Photometer, Model LI-189, LI-COR, Inc., Lincoln, USA). The relative humidity (RH, measured using a Humidity and Temperature Sensor Type HP-100-A, Umweltanalytische Mess-Systeme GmbH, Munic, Germany) ranged from 55–60%, and was maintained at roughly this level by sprinkling the floor of the glasshouse with cold water.

Propagator construction

Two low-cost poly-propagators were constructed from wooden-frames following the design of Leakey *et al.* (1990). The external parts of the frames were covered with clear, colourless polyethylene plastic. Each propagator was internally partitioned into six equal and independent compartments. Each compartment was covered with two layers of polyethylene plastic sheets, thus yielding a completely watertight unit. The compartments were then successively filled with

layers of sand (c. 1–2cm thick), stones (c. 10cm thick), small stones (c. 6cm thick) and gravel (c. 5cm thick). The topmost layer of each compartment was covered with clean sand (c. 5–7cm thick) to be used as a rooting medium. A piece of plastic pipe (diameter, 3cm; length, 40cm) was placed vertically in the front right corner of each compartment to serve as an inlet for refilling water lost through evapo-transpiration. The water table in each compartment was maintained roughly within c. 1–2cm from the bases of the cuttings. The propagators were shielded from wind by using sheets of plywood nailed onto wooden posts, and were shaded (up to 80%) by means of a 1.5m high wooden-frame covered with the leaves of *Phoenix reclinata* Jacq.

IBA preparation, cutting severance and treatment procedures

A stock solution of 3.2% indolebutyric acid (IBA) (SIGMA Chemical Company, St. Louis, MO, USA) was prepared by dissolving the hormone in a mixture of absolute ethanol and methanol in a 1:1 (v/v) ratio. Concentrations of 0.2%, 0.4%, 0.8% and 1.6% IBA were prepared from the stock solution by serial dilution with distilled water. Branch cuttings from 4- and 8-year-old stockplants were collected on the 4th week of July (a rainy month in Ethiopia) when a widespread light-green flush of juvenile leaves occurred at the tips of branchlets used for the cutting production. Cuttings from 3-month-old seedlings (entire shoots) and those from 2-year-old stockplants (branches) were collected from plants maintained in the glasshouse. Plant parts used for cuttings were harvested between 06:00am and 07:30am and were carried to the laboratory for trimming to the desired sizes (Table 1). The prepared cuttings (50–60 cuttings per treatment) were randomly allocated to each of the 0%, 0.20%, 0.40%, 0.80%, 1.60% or 3.20% IBA concentrations. The control consisted of a comparable number of cuttings treated with the solvent only. Each cutting was treated at its base with 10 μl of the corresponding IBA solution, thus resulting in 0 μg (control), 20 μg, 40 μg, 80 μg, 160 μg or 320 μg IBA/cutting, respectively. The solvent was evaporated from the treated plant parts by holding a bunch of 5–6 cuttings for c. 1 minute against a stream of cold air generated by a pump. After treatment, cuttings were packed in separate polyethylene bags and were taken to the propagators to be set in the prepared rooting medium. Approximately one-fourth the length of each cutting was inserted into the rooting medium. The experiment was run in two separate propagators (two blocks) each with six independent compartments. The six different IBA treatments (each having 50–60 cuttings) were randomly allocated to each of the six compartments of the two propa-

Table 1: Age and sizes of *Podocarpus falcatus* stockplants, cutting size-ranges and the corresponding total leaf area of cuttings used for the rooting studies

Stockplant age	Mean stockplant height ± SE	Range of cutting size		
		Cutting length (cm)	Cutting basal diameter (mm)	Total leaf area (cm ²)
3-month	10 ± 0.2	6–9	2–3	11–16
2-year	100 ± 1.4	7–11	2–3	19–26
4-year	196 ± 2.3	9–13	2.5–3.5	24–29
8-year	450 ± 8.1	10–13	2.5–3.5	28–35

gators, thus resulting in 100–120 cuttings per treatment. The relative humidity within the propagators was maintained at c. 70–80% by spraying the compartments with water twice a day. Assessments of the survival, callusing and rooting of cuttings were made seven weeks after the start of the experiment (and every two weeks thereafter). This was done by removing the cuttings from the rooting medium, checking, and setting them back in their respective places. The number of primary roots of each cutting was recorded just before transplanting the rooted cuttings to the potted soil mixture.

Seedlings vs stecklings trial

Fifty rooted cuttings derived from the 2-year-old stockplants and fifty seedlings of comparable heights and age were transplanted into plastic pots (diameter, 15cm; length, 30cm) containing a mixture of soil, horse dung, and sand, in a ratio of 2:1:1 (v/v/v), respectively. The plants were maintained in the glasshouse for 24 weeks by watering them once a day. Steckling and seedling heights were recorded at 0, 4, 8, 12, 16, 20 and 24 weeks after transplantation.

Statistical analyses

Data were analysed using STATISTICA for Windows (StatSoft, Inc. Tulsa OK, USA). ANOVA, followed by Tukey Honest Significant Difference Test, which was run for detecting significant differences among treatment means at $P \leq 0.05$. Test for ANOVA assumptions (i.e. homogeneity of variances) was run using Levene’s homogeneity test.

Results

Cuttings from 3-month-old and 2-year-old stockplants rooted well with IBA treatments between 0.20% and 0.80%, but

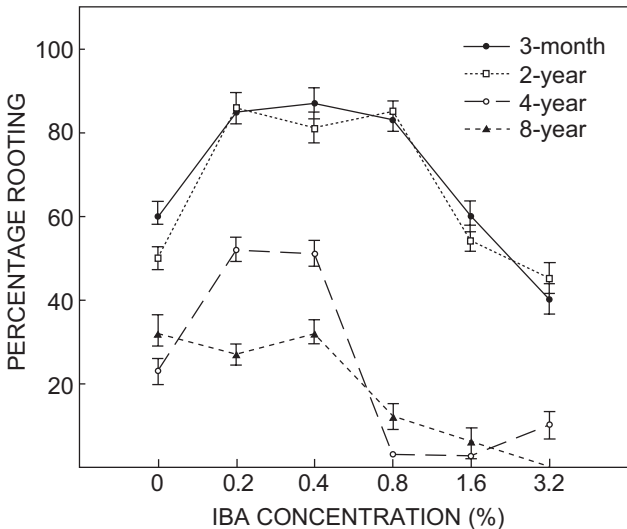


Figure 1: Impacts of various IBA concentrations on the rooting response of cuttings derived from 3-month-old, 2-year-old, 4-year-old, and 8-year-old *P. falcatus* stockplants. Bars represent \pm SE of data from two parallel experiments set up in two separate propagators (n = 50–60 cuttings per treatment)

were inhibited at higher concentrations (Figure 1). On the other hand, IBA levels higher than 0.40% significantly ($P < 0.01$) inhibited rooting in cuttings derived from 4-year-old and 8-year-old stockplants. All IBA treatments to cuttings from the 3-month-old and 2-year-old stockplants significantly ($P < 0.01$) reduced the time taken for the 50% rooting response (Table 2).

Rooting progress of cuttings derived from different age-categories of donor plants and treated with 40 μ g IBA/cutting is shown in Figure 2. Rooting in cuttings from 3-month-old and 2-year-old stockplants was significantly ($P < 0.01$) greater than in those from 4-year-old and 8-year-old stockplants. The difference in the final rooting response between cuttings derived from 4-year-old and 8-year-old stockplants was significant ($P < 0.01$), but the corresponding difference between the 3-month-old and 2-year-old stockplants was not (Figure 2).

Root numbers were significantly ($P < 0.01$) greater in cuttings derived from older stockplants than in those from younger stockplants, irrespective of IBA treatments (Figure

Table 2: Time taken for the 50% rooting response of *Podocarpus falcatus* cuttings. The cuttings were obtained from 3-month-old and 2-year-old stockplants and were treated with 10 μ l of various IBA concentrations

IBA concentration (%)	Stockplant age	
	3-month-old	2-year-old
0.0	18 weeks	19 weeks
0.2	11 weeks	11 weeks
0.4	13 weeks	13 weeks
0.8	9 weeks	12 weeks
1.6	9 weeks	12 weeks
3.2	11 weeks	14 weeks

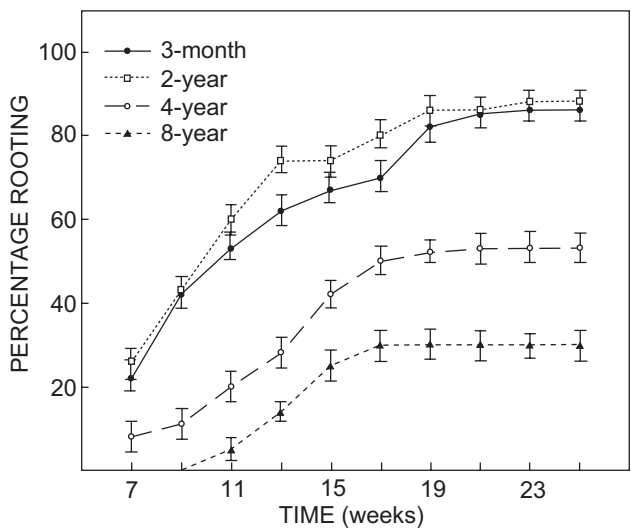


Figure 2: Progress of rooting in cuttings derived from 3-month-old, 2-year-old, 4-year-old, and 8-year-old *P. falcatus* stockplants treated with 10 μ l of 0.4% IBA cutting⁻¹. Bars represent \pm SE of data from two parallel experiments set up in two separate propagators (n = 50–60 cuttings per treatment)

3). Whereas IBA treatments at 0.4% and 0.8% increased root numbers significantly ($P < 0.01$) in cuttings from younger stockplants, no similar impacts were observed in those from older stockplants (Figure 3). Typical rooting responses in cuttings derived from 4-year-old and 8-year-old stockplants are shown in Figure 4.

Stecklings grew significantly ($P < 0.01$) faster than seedlings, especially from the 8th week onwards after transplantation (Figure 5). Within stecklings themselves, those with larger root numbers grew significantly ($P < 0.01$) faster than those with smaller root numbers.

Discussion

Impact of stockplant age on rooting

Studies on coniferous trees have shown that rooting of cuttings from physiologically mature stockplants (in some species as young as 1-year-old) has been rather difficult (e.g. Talbert *et al.* 1993, Haissig and Davis 1994, Berhe and Negash 1998, Negash 2002b). Rooting success in *Robinia pseudoacacia* and *Grewia optiva* was reported to be better in cuttings derived from juvenile than from mature stockplants (Swamy *et al.* 2002). A survey on the rooting ability of 100 tropical rainforest tree species showed a declining trend in rooting capacity as the age of donor plants increased (Itoh *et al.* 2002).

Leahey *et al.* (1992) attributed rooting ability of cuttings to the influence of 'physiological ageing' of the donor plant. Hartmann *et al.* (1990) emphasised the importance of 'biological' age (rather than 'chronological' age) on rooting success of woody plants. Studies on *P. falcatus* and *Juniperus procera* showed age of stockplants as one of the most important factors controlling adventitious rooting (Semagn and Negash 1996, Berhe and Negash 1998, Negash 2002b). Haissig and Davis (1994) commented that even

though the ability to control maturation of woody species would be of immense economic benefit in forestry and horticulture, neither empirical trials to modify maturation nor physiological research to understand maturation has made significant progress to date.

Several explanations have been offered for the limited rooting response of cuttings derived from mature stockplants. Some of the suggested reasons include decreased sensitivity of mature tissues to root-promoting substances (Zajczkowski 1973, Hartmann *et al.* 1990), decline in auxin content with age (Hartmann *et al.* 1990), development of lignified tissues that slow down or prevent morphological changes (White and Lovell 1984a), presence of large numbers of resin canals, sclerenchymatous cells, and branch traces (White and Lovell 1984b), increased production of rooting inhibitors or lowered phenolic levels that act as auxin co-factors (Hartmann *et al.* 1990), and physiological ageing (Leahey *et al.* 1992).

This study found out that up to 80% of cuttings derived from juvenile seedlings (3-month-old and 2-year-old) rooted

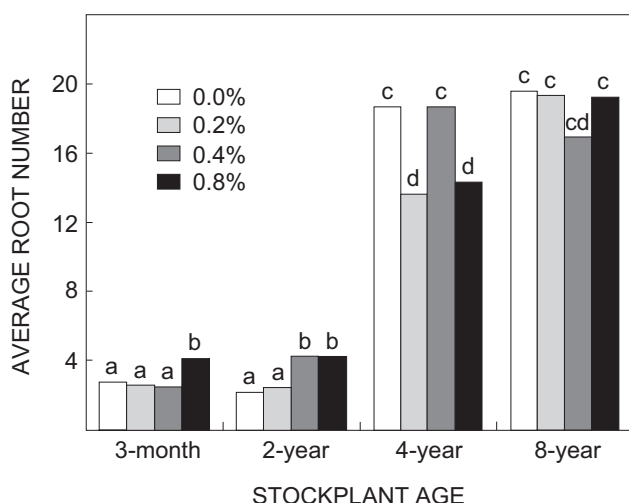


Figure 3: Effects of various IBA concentrations on root numbers of cuttings derived from 3-month-old, 2-year-old, 4-year-old, and 8-year-old *P. falcatus* stockplants. Means labelled with the same letter are not significantly different from each other at $P \leq 0.05$



Figure 4: Typical rooting response of cuttings treated with 10 µl of 0.4% IBA/cutting and derived from 4-year-old (left) and 8-year-old (right) stockplants. The scale is 20cm

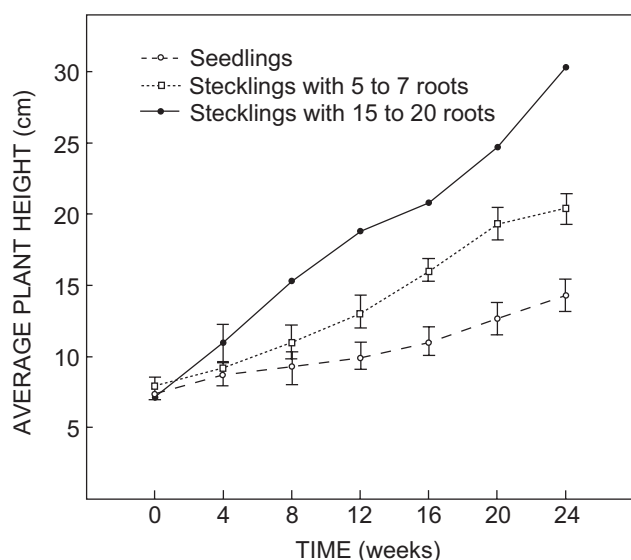


Figure 5: Growth of seedlings and stecklings (plants derived from rooted cuttings) of *P. falcatus*. Bars represent \pm SE ($n = 50$)

successfully, but the rate of rooting was relatively slow (Figures 1 and 2). The main drawback with 3-month-old stockplants was that only one cutting per seedling could be obtained, but the rooted cuttings grew faster than the corresponding seedlings probably due to the large root numbers possessed by the former. Morphologically, the stecklings from these juvenile stockplants were similar to seedlings and were free from plagiotropism (gravitational response leading to branch-like growth habit). That cuttings from juvenile donor plants root relatively easily and are indistinguishable from seedlings has been reported by a number of workers across a wide range of tree species (Talbert *et al.* 1993, Ritchie 1994).

Effect of IBA on rooting

Indolebutyric acid has been widely used for rooting various tropical tree cuttings (e.g. Mesén *et al.* 1997, Berhe and Negash 1998, Negash 2002b) and a wide range of non-tropical species (e.g. Hartmann *et al.* 1990, Davis and Haissig 1994). Data shown in Figure 1 demonstrate that cuttings derived from younger stockplants responded better to IBA dosages between 20 μ g and 80 μ g per cutting than those from the older ones. A number of investigators have reported that optimal IBA concentration varies from species to species, and is affected by stockplant pretreatment (Howard 1994, Leakey *et al.* 1994, Copes and Mandel 2000, Mesén *et al.* 2001, Itoh *et al.* 2002), size and basal diameter of cuttings (Leakey *et al.* 1994, Negash 2002b), position of cuttings on stockplants and number of leaves on cuttings (Hartmann *et al.* 1990, Leakey *et al.* 1994). Consequently, reproducible results have not been easy to achieve, possibly because adventitious root formation is a synchronised developmental process involving various biochemical, physiological and histological events in the induction, initiation, and elongation of root primordia (Davies and Hartmann

1988, Loach 1988, Hackett and Murray 1994).

Growth of stecklings was significantly greater than that of seedlings, perhaps because stecklings possessed significantly more roots than seedlings of comparable age and size. Consequently, stecklings were able to acquire water and nutrients from the soil more efficiently than the corresponding seedlings. According to Ritchie (1994), trees grown from stecklings in Australia yielded substantially more biomass than those from seedlings of the same age. Similarly, Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) from stecklings were more uniform, faster growing, and were more resistant to pests than trees from seedlings (Minghe and Ritchie 1999a, 1999b). On the other hand, Talbert *et al.* (1993) mentioned studies on *Pinus radiata* D. Don in which stem taper, bark thickness, sweep and branch diameter were found to be less on cutting-origin plants than on seedling-origin ones when the cuttings were taken from trees that were five or more years old.

From the study in this paper, it was concluded that cuttings derived from juvenile stockplants of East African yellowwood rooted reasonably well, but the rate of rooting was relatively slow. The observation that branch cuttings (with light-green flush leaves) derived from the more mature stockplants have rooted (albeit poorly) provides opportunities for establishing clones from mature elite trees of desirable biological and/or economic attributes. However, further studies are needed to examine the impacts of various biological, chemical and physical factors on the adventitious rooting of this graceful, yet threatened, tree species. It is also suggested that studies directed towards controlling plagiotropism in stecklings derived from mature stockplants would be of considerable practical importance.

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